NEW HETEROCYCLIC COMPOUNDS, METHOD OF DEVELOPING NEW DRUG LEADS AND COMBINATORIAL LIBRARIES USED IN SUCH METHOD

FIELD OF THE INVENTION

This invention is in the field of using combinatorial chemistry to develop new drugs.

LIST OF REFERENCES

The following references are considered to be pertinent for the purpose of understanding the background of the present invention:

Adang A.E.P. and Hermkens P.H.H., *Curr. Med. Chem.* **8**:985 (2001);

Beeley N.R.A., *Drug Disc. Today* **5**:354 (2000);

Bunin B.A. and Ellman J.A., *J. Am. Chem. Soc.* **114**:11997 (1992);

Campian E. et al, *Bioorg. Med. Chem Lett.* **8**:2357(1998);

Furka A. et al. *Int. J. Pept. Protein. Res.* **37**:487-493 (1991);

Geysen H.M. et al. *Proc. Natl. USA*, **81**: 3998 (1984);

Hougten R.A., *Proc. Natl. USA*, **82**:5131 (1985);

Kumar S. et al. *Prot. Sci.* **9**:10-19 (2000);

Lipinsky C.A. et al., *Adv. Drug Deliv. Rev.* 23, 3 (1997);

Lipinsky C.A., *Chimia* **52**:503 (1998);

Morrison K.L. and Weiss G.A., *Curr. Opin. Chem. Biol.* **5**:302-307 (2001);

Rink H., *Tetrahedron Lett.* **28**: 3787 (1987);

Winter et al. *Nature* **299**: 756 (1982);

The above references will be acknowledged in the text below by indicating the author's name and year of publication (in brackets) from the above list.

BACKGROUND OF THE INVENTION

An important objective of combinatorial chemistry is to generate a large number of novel compounds that can be screened to identify lead compounds for pharmaceutical research. Theoretically, the total number of compounds which may be produced for a given library is limited only by the number of reagents available to form substituents on the variable positions on the library's molecular scaffold.

The combinatorial process lends itself to automation, both in the generation of compounds and in their biological screening, thereby greatly enhancing the opportunity and efficiency of drug discovery. Combinatorial chemistry may be performed in a manner where libraries of compounds are generated as mixtures, while the complete identification of the individual compounds is postponed until after positive screening results are obtained. However, a preferred form of combinatorial chemistry is "parallel array synthesis", (also called Multiple Parallel Synthesis, MPS) where individual reaction products are simultaneously synthesized, but are retained in separate compartments. [Geysen et al. (1984); Hougten (1985)]. For example, the individual library compounds can be prepared, stored, and assayed in separate wells of a microtiter plate, each well containing one member of the parallel array. The use of standardized microtiter plates or equivalent apparatus, is advantageous because such an apparatus is readily accessed by programmed robotic machinery, both during library synthesis and during library sampling or assaying.

Combinatorial chemistry can be carried out in solution phase where both reactants are dissolved in solution or in solid phase where one of the reactants is covalently bound to a solid support. Solid phase synthesis offers the advantage that reactions can be carried out using excess reagents, while the solid support-bound products are easily washed free of excess reagent. The use of

excess reagents may ensure high yield of each step in a multiple step synthesis. Solution phase synthesis typically requires use of one or more reaction mixture work-up procedures to separate reaction product from unreacted excess reagent.

The first combinatorial libraries were composed of peptides, in which all or selected amino acid positions were randomized [Geysen et al. (1984); Furka et al. (1991)].

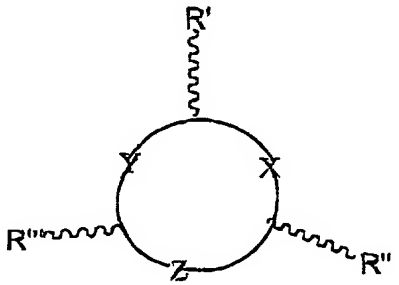
Peptides and proteins can exhibit high and specific binding activity, and can act as catalysts. In consequence, they are of great importance in biological systems. Unfortunately, peptides per se have limited utility for use as therapeutic entities. They are costly to synthesize, unstable in the presence of proteases, non selective and in general do not pass cellular membranes.

Nucleic acids have also been used in combinatorial libraries. Their great advantage is the ease with which a nucleic acid with appropriate binding activity can be amplified. As a result, combinatorial libraries composed of nucleic acids can be of low redundancy and hence, of high diversity. However, the resulting oligonucleotides are not suitable as drugs for several reasons. First, the oligonucleotides have high molecular weights and cannot be synthesized conveniently in large quantities. Second, because oligonucleotides are polyanions, they do not cross cell membranes. Finally, deoxy- and ribo-nucleotides are hydrolytically digested by nucleases that occur in all living systems and are therefore usually decomposed before reaching the target.

There has therefore been much interest in combinatorial libraries based on small molecules (i.e. molecules having molecular weight of up to about 1000 daltons), which are more suited to pharmaceutical use, especially those which, like benzodiazepines, belong to a chemical class which has already yielded useful pharmacological agents [Bunin and Ellman (1992); Beeley (2000)]. The techniques of combinatorial chemistry have been recognized as the most efficient means for finding small molecules that act on these targets. At present, small molecule combinatorial chemistry involves the synthesis of either pooled or discrete molecules that present varying arrays of functionality on a common

scaffold. These compounds are grouped in libraries that are then screened against the target of interest either for binding or for inhibition of biological activity [Adang and Hermkens (2001)].

The elements of diversity of libraries of currently available scaffold based compounds having the general structure **(A)** showed below, are based mainly on sequential or positional diversity namely the order in which the various R groups are arranged around the ring and chemical diversity that can arise from alterations in the chemical nature of the R groups.



(A)

In the above structure **(A)**, X, Y and Z represent ring heteroatoms or carbons, and R', R'' and R''' represent substituents associated to the ring through a linker (showed schematically as a wavy line).

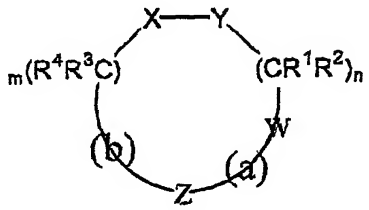
It is known from the art [Kumar S. et al. (2000)] that molecules may bind to each other if their conformations are complementary in geometry and chemistry and if their binding produces stable associations. However, most of the known libraries of organic molecules suffer from a major drawback when applied for the discovery of new drug leads based on the inhibition of peptide: protein or protein: protein or protein: nucleic acid interactions: they are too constrained and therefore lack the ability to undergo conformational complementarity, i.e. lack an ability for binding to a protein and/or a nucleic acid. This led to the preparations of extremely large libraries (consist of up to millions of compounds) and their biological screening, which in many cases

results in the discovery of low affinity leads or to the lack of their discovery.

SUMMARY OF THE INVENTION

The present invention provides, according to a first of its aspects, new compounds that have a relatively flexible scaffold. These compounds may be used to produce new combinatorial libraries that will permit, e.g. in high throughput screening assays, to screen for and select drug candidates for a variety of uses in human medicine, veterinary medicine and in agriculture. The members of each library according to the invention differ from each other in the ring size, in addition to the conventional chemical and positional diversity attained by the different substituents on the scaffold, thus allowing the selection of the most active compound, not only on the basis of the nature and proper arrangement of the substituents (attained by the known chemical and positional diversity), but also based on the ability to undergo conformational complementarity (attained by the conformational diversity).

Thus, the present invention provides scaffold based compounds having the following general formula (B):



(B)

including pharmaceutically acceptable salts, esters or solvates thereof,

wherein

Z is selected from C=O and -CH_2-,

W is selected from C=O and a bond, provided that at least one of Z and W is C=O,

X and Y are independently selected from CH_2, O, S, NH, N-R^5, NH-CO, CO, CH_2CO, S=O and SO_2, or X and Y may form together a group selected from CH=CH, CO-NR^5, NH-CO-NH, O-CH(R^5)-O, NH-CH(R^5)-O and NH-CH(R^5)-NH, where the hydrogen in the above groups may optionally be substituted by an alkyl group;

(a) and **(b)** are parts of the scaffold and are nitrogen containing bivalent organic radicals, each independently providing between 1 to 4 , preferably up to 2 atoms to said scaffold,

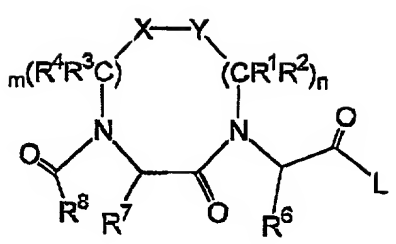
R^1, R^2, R^3 and R^4 are each independently selected from H and substituted or unsubstituted alkyl, and

n and m are integers from 1 to 6.

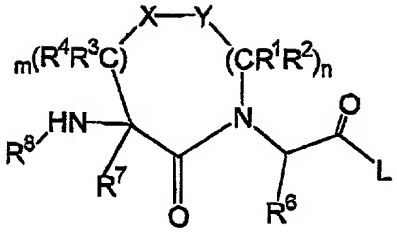
Preferably, the present invention provides scaffold based compounds having the formula **(B)** above, wherein X and Y are independently selected from CH_2, O, S, NH, N-R^5, NH-CO, CO, CH_2CO, S=O and SO_2, or X and Y may form together a group selected from CO-NR^5, NH-CO-NH, O-CH(R^5)-O, NH-CH(R^5)-O and NH-CH(R^5)-NH, where the hydrogen in the above groups may optionally be substituted by an alkyl group.

According to a preferred embodiment, each of parts (a) and (b) is independently selected from -N(CHR^6CO-L)-, - C(CO-L)(R^6)-, -N(COR^8)-CHR^7-, and -C(NHR^8)(R^7)-.

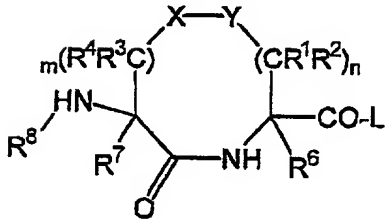
According to another preferred embodiment, the present invention provides heterocyclic compounds having the formula **I, II, III** or **IV**:



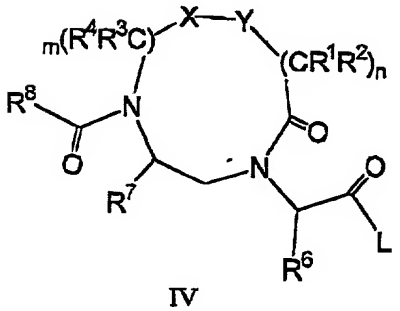
I



II



III



IV

including pharmaceutically acceptable salts, esters or solvates thereof,

wherein

X and Y are independently selected from CH_2, O, S, NH, N-R^5, NH-CO, CO, CH_2CO, S=O and SO_2, or X and Y may form together a group selected from CH=CH, CO-NR^5, NH-CO-NH, O-$CH(R^5)$-O, NH-$CH(R^5)$-O and NH-$CH(R^5)$-NH, where the hydrogen in the above groups may optionally be substituted by an alkyl group;

R^1, R^2, $R^3$and R^4 are each independently selected from H and substituted or unsubstituted alkyl,

R^5, R^6, R^7 and R^8 are each independently selected from H, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, hetroaryl, heterocyclyl, hetroaralkyl, acyl, carboxyaryl, carboxyalkyl, side chains of naturally and artificially occurring amino acids as well as derivatives and mimics of such side chains, and linear or cyclic peptide;

L is selected from H, OH, NH_2, NHR^5, a peptide and a solid support, where R^5 is as defined above, and

n and m are integers from 1 to 6, with the exclusion of the following compound: glycinamide, L-tyrosyl-N-[2-[(2S)-4-[(1S)-1-carboxy-3-methylbutyl]-3,4,5,8-tetrahydro-3-oxo-2-(phenymethyl)-1,4-diazocin-1(2H)-yl]2-oxoethyl.

According to another preferred embodiment, in the above compounds of formulae **I, II, III** and **IV**, X and Y may form together a group selected from CO-NR^5, NH-CO-NH, O-$CH(R^5)$-O, NH-$CH(R^5)$-O and NH-$CH(R^5)$-NH, where the hydrogen in the above groups may be substituted by an alkyl group.

The following combinations for X and Y are preferred: both X and Y are S; or X is NH and Y is CO; or X is CO and Y is NH; or X is NH and Y is CH_2; or X is CH_2 and Y is NH; or X and Y are NH-CO-NH; or X is N-R^4 and Y is CO; or (vi) X is CO and Y is N-R^4.

The invention also provides, according to another of its aspects, a combinatorial library comprising two or more, preferably a plurality, of compounds of any one of the formulae **(B), I, II, III** or **IV**. The library of the invention serves as a readily accessible source of diverse macrocyclic compounds for use in identifying new biologically active macrocyclic compounds through pharmaceutical and veterinary candidate screening assays, for the development of highly effective and environmentally friendly insect control and crop control agents, for use in studies defining structure/activity relationships, and/or for use in clinical investigation.

The selection of an active candidate is preferably achieved from a library of compounds that have the same substituents in the same positions along the scaffold but the scaffolds differ from each other in size and chirality of the substituents and therefore in their conformation. The libraries are prepared by the multiple simultaneous solid phase method [Hougten R.A.,1985] or its automated version, and contain the calculated number of diversity possibilities. Libraries are typically synthesized in a 12-48 format, namely each library typically contains 12-48 members. Each member of the library will be characterized, purified and subjected to biological assay. The present invention also provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an effective

amount of a compound of the formula **I, II, III** or **IV,** as defined above. The new compounds of the invention may act as modulators of the activity of cells which activity is mediated by proteins or peptides as will be explained below.

The term *"effective amount"* refers to an amount capable of decreasing, to a measurable effect, at least one adverse manifestation of the disease and should be chosen in accordance with the drug used, the mode of administration, the age and weight of the patient, the severity of the disease, etc.

Also provided by the invention is use of a compound of the formula **I, II, III** or **IV,** as defined above, for the preparation of a pharmaceutical composition.

In addition, the present invention provides a method for modulating a protein or peptide-mediated cell activity, such method comprising contacting a compound of the formula **I, II, III** or **IV,** as defined above, with a cell or cellular component having said peptide or protein. The cell may be from an eukaryotic or prokaryotic organism, from a uni- or multi-cellular organism and may be from plant, bacteria or animal. The cellular component is selected from cellular organells such as nucleous, ribosomes, mitochondria and cell membranes or from cellular molecules such as receptors, enzymes, substrates, ligands and the like.

Examples of peptide or protein-mediated cell activities which are modulated by the compounds of the invention are: proliferation, differentiation, cellular shape alteration, cellular elongation, uptake of substances by cells (glucose, neurotransmitters), secretion of substances, cellular metabolism, expression of various proteins.

Also provided by the present invention, a method for the treatment of a disease, disorder or condition wherein a therapeutically beneficial effect may be evident by the modulation of a protein or peptide-mediated cell activity, the method comprising: administering to a subject in need of such treatment a therapeutically effective amount of a compound of formula **I, II, III** or **IV.**

In accordance with another embodiment of the invention, the compound of the invention may be bound to a detectable label such as a fluorescence-emitting moiety, a radio-label, a label capable of undergoing an enzymatic reaction

producing a detectable color, a marker for x-ray, MRI, radio-isotope imaging or PET scan, to produce a labeled adduct. Then, upon administration of such labeled adduct, it may be detected at a desired location by any manner known in the art and in accordance with the specific label used, for example, fluorescence, radioactive emission, or a color production, MRI, x-ray and the like.

The term *"bound"* refers to covalent or non-covalent (e.g., electrostatic) binding, which connects the compound of the invention to the detectable label. Alternatively, the compound of the invention may have inherent detectable properties of its own, that enable it to be detected by any of the above mentioned techniques.

The present invention is also directed, according to a further aspect thereof, to a method for designing new compound libraries that have novel type of structural complexity and diversity, and can be screened to identify potent modulators of protein or peptide-mediated cell activity, so as to develop lead compounds for pharmaceutical, veterinary or agricultural research. Molecules having a molecular weight of up to about 1000 daltons, i.e. small molecules, are preferable.

More specifically, the present invention provides a method of identifying a candidate, which modulates a protein or peptide-mediated cell activity, the method comprising:

(a) identifying in said protein or peptide, a domain which is essential for said activity,

(b) identifying in said domain, pharmacophors essential for the activity,

(c) planning a combinatorial library of cyclic compounds having the formula **I, II, III** or **IV**, as defined above, wherein each such compound comprises at least two of the pharmacophors identified in step (b) above or mimics or derivatives of the pharmacophors, where each member of the library differs from the other by at least one of the following: i) the size of the ring; ii) the order in which the pharmacophors are arranged in the ring; iii) the chemical nature of the ring; iv) the chemical nature of the pharmacophors; v) the

chirality of the linker between the ring and the pharmacophor; and vi) the chirality of the pharmacophor,

(d) synthesizing a plurality of compounds, such compounds being among the compounds of the combinatorial library planned in step (c);

(e) screening the synthesized compounds of step (d) for candidates that modulate said activity.

The term *pharmacophor* refers to the ensemble of steric and electronic features that is necessary to ensure the optimal molecular interactions with a specific biological target structure and to trigger (or to block) its biological response. In the present invention the term refers to those moieties of the side chain or backbone of the peptide or protein (which mediates the cell activity), that are necessary for the binding to the other cellular components, the binding eliciting a biological response. The pharmacophor may be a chemical moiety present on a single side chain or a collection of chemical moieties present in spatially adjacent side chains.

The above method may also be used in order to identify a compound which modulates a protein or peptide-mediated cell activity. In such case the method comprises the following additional steps after step (e):

(f) collecting those compounds which modulate said activity in a test assay as compared to the modulation in the same test assay in the absence of said compound, thereby obtaining modulators of a protein or peptide-mediated cell activity; and

(g) producing the compounds selected in step (f) above.

The present invention further provides a compound which modulates a protein or peptide-mediated cell activity obtained by the above method.

The present invention may also be utilized in agriculture. Therefore, the present invention also provides a method for the discovery of new agents for use in agriculture, wherein such agents are based on modulators of proteins or

peptides derived from insects or plants.

GLOSSARY

Definitions

A *"library"* is a collection of compounds which, while sharing some common structural elements (which may be common scaffolds, common ring sizes, common substituents and the like), are diverse from each other by at least one of the following properties: i) the size of the ring; ii) the order in which the pharmacophors are arranged in the ring; iii) the chemical nature of the ring; iv) the chemical nature of the pharmacophors; v) the chirality of the linker between the ring and the pharmacophor; and vi) the chirality of the pharmacophor. The library allows screening from among a plurality of compounds for those that have a desired property. The library may be designed by a combinatorial or classical chemical process.

A *"lead compound"* is a library compound in a selected combinatorial library, for which the assay has revealed significant effect relevant to a desired cell activity to be modulated. In the present case the property is the modulation of at least one peptide or protein-mediated activity.

"Peptide or protein-mediated cell activity" refers to a physiological property of a cell that is caused, directly or indirectly (the latter referring to an effect caused by an effector which is more downstream in the pathway) by the interaction between a protein or peptide and another cellular component (the term "cellular component" including: other proteins or peptides of the same or different types, membranes, nucleic acids, lipoproteins, nucleotides, co-factors, hormones, ion effectors and the like). The interaction between the protein and the other cellular component may be of the type: receptor-ligand, enzyme-substrate, DNA-binding proteins – DNA etc. Said interaction mediates (causes) directly or indirectly a cell activity such as: expression of a protein, proliferation, differentiation, cell-elongation, cell-shape alteration, cellular metabolism, cellular update of external substances, secretion of substances from the cells and the like.

"Modulate/Modulator" refers to increase or decrease in at least one peptide or protein-mediated cell activity, in the presence of the compound of the invention, or to the change of the response of the cell to the presence of a physiological cue ,as compared to the activity or response, respectively, in the absence of the compound. Examples of such physiological cues are presence of effectors, the modulation being a change in the cellular response to a ligand, hormone, response to toxic substances (pesticides), stress (heat shock, draught, lack of nutrients) aging and the like.

The term ***"substituents"*** refers to chemical radicals which are bonded to or incorporated onto the scaffold through the synthesis process of the library. The different functional groups account for the diversity of the molecules throughout the library and are selected to impart diversity of structure, function and biological activity to the scaffold in the case of diverse libraries, and optimization of a particular biological activity in the case of directed libraries.

"Aryl" means one or more aromatic rings, each of 5 or 6 ring carbon atoms and includes substituted aryl having one or more non-interfering substituents. Multiple aryl rings may be fused, as in naphthyl, or unfused, as in biphenyl.

"Alkyl" means straight or branched chain or cyclic hydrocarbon having 1 to 10 carbon atoms.

"Substituted alkyl", is alkyl having one or more non-interfering substituents.

"Halo" means chloro, fluoro, iodo or bromo.

"Heterocycle" or ***"heterocyclic"*** means one or more rings of 5, 6 or 7 atoms with or without unsaturation or aromatic character, optionally substituted with one or more non-interfering substituents, and at least one ring atom which is not carbon. Preferred heteroatoms include sulfur, oxygen, and nitrogen. Multiple rings may be fused, as in quinoline or benzofuran, or unfused as in 4-phenylpyridine. Suitable substituents on the heterocyclic ring structure include,

but are not limited to halo, Cl-C10 alkyl, C2-Cl0 alkenyl, C2-Cl0 alkynyl, Cl-Cl0 alkoxy, C7-C12 aralkyl, C7-C12 alkaryl, Cl-Cl0 alkylthio, arylthio, aryloxy, arylamino, C3-C10 cycloalkyl, C3-C10 cycloalkenyl, di(C1-C10)-alkylamino, C2-C12 alkoxyalkyl, Cl-C6 alkylsulfinyl, Cl-Cl0 alkylsulfonyl, arylsulfonyl,aryl, hydroxy, hydroxy(Cl-C10)alkyl, aryloxy(Cl-C10)alkyl, C1-Cl0 alkoxycarbonyl, aryloxycarbonyl, aryloyloxy, substituted alkoxy, fluoroalkyl, nitro, cyano, cyano(C1-C10)alkyl, Cl-Cl0 alkanamido, aryloylamido, arylaminosulfonyl, sulfonamido, amidino, carbamido, carboxy, heterocyclic radical, nitroalkyl, and $-(CH_2)_m$-Z-(C1-C10 alkyl), where m is 1 to 8 and z is oxygen or sulfur.

The term ***"solid support"*** refers to a solvent insoluble material having cleavable covalent bonds for use in preparing the library compounds of the invention.

DETAILED DESCRIPTION OF THE INVENTION

Many biological processes are critically dependent on protein:protein, protein:peptide and protein:nucleic acid interactions, and many drugs are small molecules known to disrupt such interactions (antagonists) or alternatively mimic one component of the interaction in such a manner so that the activity controlled by the interaction can take place in the presence of the drug and the other cellular component (agonist).

The drugs which work by interruption of such interactions (for example by the interruption of a receptor-ligand interaction) mimic in fact a domain of one of the proteins participating in the interaction. By one option this mimic creates an antagonist that competes with the protein for binding with the other member of the interaction (the other cellular component), leading to decrease in the interaction and hence decrease of the cell activity controlled or caused (directly or indirectly) by the interaction.. Where the cell activity is an *"on"* physiological process, (for example, increase in production of an agent), the interruption will close the *"on"* reaction and decrease the physiological process (decrease production of the agent). Where the cell activity is an *"off"* reaction (for example a signal causing inhibition of proliferation) the interruption will close the *"off"* reaction and will increase the physiological process, for example, cause increased proliferation.

By another option the drug may work as an agonist and cause the modulation of the cell activity by mimicking the protein (that is essential for the cell activity) in the interaction in such a manner that the cell activity takes place as if the native protein and not the compound were interaction with the other cellular component.. For example the compound may be able to activate the cellular component with which the protein interacts in a similar way to the protein itself.

The aim of the compounds of the present invention is to mimic a region in one of the participators of the interaction, so as to compete for the binding on the

other participator of the interaction (either in the antagonist or the agonist manner) thus changing the interaction and leading to a change in the physiological process or property controlled by the interaction.

The rational for the present invention is the following: many libraries used for the discovery of drug leads are composed of heterocyclic scaffolds that are too constrained (rigid) to allow conformational complementarity essential for the interactions with proteins, peptides, polysaccharides or nucleic acids. The combinatorial library of the invention allows the generation of sub-libraries with spatial diversity, which is obtained by the diversity in ring size and chirality of the link between the substituent and the scaffold.. This results in a library where each individual scaffold has a different flexibility and a different spatial positioning of the pharmacophor. The design of the library of the invention increases the probability that some members of the library have the ability to undergo conformational complementarily, i.e. the pharmacophors are present in the correct orientation to interrupt or mimic the interaction with the other cellular component. The present invention allows the design and synthesis of libraries which occupy a larger proportion of the "probability space" of the pharmacophor positioning (i.e. increase the probability of the substituents to be present in varying positions in the space, thus increasing the probability that at least one positioning-conformation is the bioactive conformation,) while still creating relatively small, focused libraries. These properties lead to fast discovery and optimization of novel drug leads.

The classical elements of diversity of state-of-the-art, currently available macrocyclic, i.e. scaffold based libraries are based mainly on:

(1)The chemical nature of the scaffold;

(2)The size and chemical nature of the linkers that connect between the scaffold and the various substituents;

(3)The chemical nature of the substituents;

(4)The order in which the substituents are arranged on the scaffold.

The libraries of the new compounds of the present invention comprise a novel element of diversity, namely spatial diversity, that results from the varying size of the scaffold ring, the chirality of the linker or from a combination of the two. This diversity is new in the field of small molecule combinatorial chemistry.

Spatial diversity is defined as diversity elements that alter the conformation of the compounds, which in fact lead to altered spatial positioning of the pharmacophors. The present invention deals with libraries of compounds having new elements of diversity, namely spatial diversity elements: the chirality of the linkers (which effects the spatial arrangement of the substituents) and the size of the ring. The members of the library of the invention may also differ from each other in the classical elements of diversity (1)-(4) mentioned above.

According to a specific embodiment of the present invention, all the members in a library have the same elements of diversity (1)-(4) mentioned above: the same scaffold with the same composition and order of atoms within the scaffold; the same linker with the same size and chemical nature; the same substituents/pharmacophors arranged in the same order on the scaffold, but they differ from each other in the size of the scaffold and/or the chirality of the linker. This in turn determines the possible conformational (spatial) positioning of the pharmacophor of each compound and allows for the selection of the lead compound having the appropriate ability of conformational complementarity. The libraries of the invention are composed of a series of compounds that differ from each other by an incremental alteration of their possible conformations. Thus, every library covers an entire range of the conformational probabilities and increases the chances of obtaining a compound with at least one bioactive conformation.

Design of the libraries

The method of the present invention, for identifying a candidate which modulates a protein or peptide-mediated cell activity, comprises the following steps:

(a) identifying in said protein or peptide, a domain essential for said activity,

(b) identifying in said domain, pharmacophors essential for the activity,

(c) planning a combinatorial library of cyclic compounds having the formula **I, II, III** or **IV** as defined above, wherein each such compound comprises at least two of the pharmacophors identified in step (b) above or mimics or derivatives of the pharmacophors, where each member of the library differs from the other by at least one of the following: i) the size of the ring; ii) the order in which the pharmacophors are arranged in the ring; iii) the chemical nature of the ring; iv) the chemical nature of the pharmacophors; v) the chirality of the linker between the ring and the pharmacophor; and vi) the chirality of the pharmacophor;

(d) synthesizing a plurality of compounds from the combinatorial library planned in step (c);

(e) screening the compounds synthesized in step (d) for candidates that modulate said activity.

Between the step (c) of planning of the full library and step (d) of synthesizing a plurality of compounds from the library (which may form together the full library or a part of the library), it is possible to add a step of virtually screening the library to identify those compounds which are better mimics of the domain than others. Such virtual screening can help and predict

which compounds have a better chance of assuming the bioactive conformation and it is preferable to start the screening with the compounds that are, according to 3D modeling the most likely mimics.

The above steps are showed schematically in the following chart:

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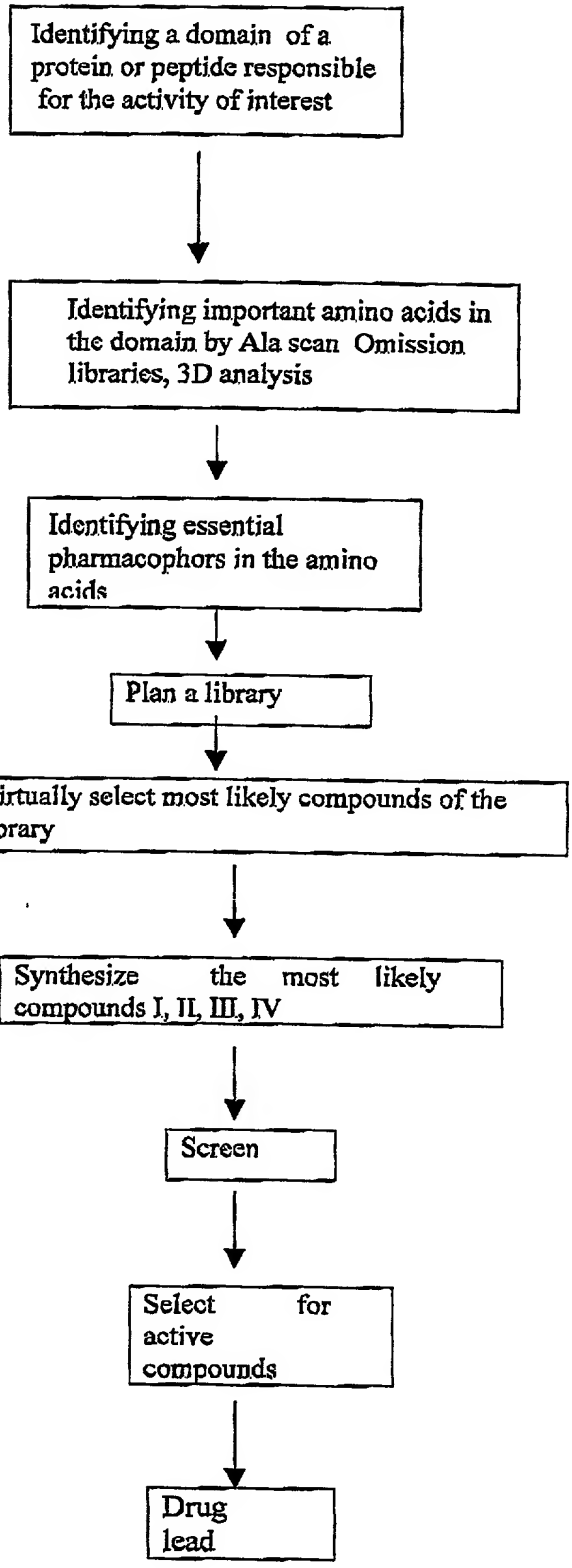

Identifying a domain of a protein or peptide responsible for the activity of interest
Identifying important amino acids in the domain by Ala scan Omission libraries, 3D analysis
Identifying essential pharmacophors in the amino acids
Plan a library
Virtually select most likely compounds of the library
Synthesize the most likely compounds I, II, III, IV
Screen
Select for active compounds
Drug lead

As the compounds of the invention intend to mimic a domain in a protein, so as to interrupt or to mimic its interaction with other cellular components and thus modulate the cell activity (mediated by the protein), it is desired that they resemble the desired domain and the positioning of the pharmacophors in the domain of the protein (which pharmacophors they mimic) as close as possible. Therefore, when deciding at the library planning step how to produce the best library, and at the synthesizing step, which of the members of the planned library should be synthesized, the following questions should be asked:

1. Are the relevant pharmacophors (or derivatives or mimics of the pharmacophors) present in the planned library?
2. Is the order of the pharmacophors on the scaffold and the distance of the pharmacophores from each other, suitable for achieving a suitable bioactive conformation (correct positioning of the pharmacophor)?
3. Is the compound capable in one of its conformation of attaining the correct positioning of the pharmacophors?
4. Is the possible conformation energetically favorable?
5. Is there a certain degree of conformation flexibility to allow conformational complementarity?

Most of the above questions can be answered during the planning stage and the synthesis decision stage on a computer using commercially available bioinformatic programs such as Tripose™.

The coordinates of amino acid side chains of a protein can be obtained from the Protein Data Bank (PDB) files. This data is based on the 3D structure of the protein (preferably as a complex with the appropriate ligand) either

obtained by crystallography or homology modeling. The 3D information allows to identify the exposed side chains and these accesible side chains are possible pharmacophors. In cases of proteins for which the 3D structure was not determined, the essential amino acids within a protein may be determined by the method of combinatorial alanine–scanning (Morrison and Weiss, (2001) *Curr. Opin. Chem. Biol.* **5**, 302-307), also known as Ala-Scan. Another method is known as omission libraries and is described in Campian et al. (1998) *Bioorg. Med. Chem Lett.* **8**, 2357. Yet other methods are site directed mutagenesis and protein engineering (Winter et al (1982)). The amino acids and backbone elements essential for a certain function may be divided into two categories: those who interact with the receptive protein, nucleic acid, polysaccharide or cell membrane and those responsible for the conformation of the essential region. The side chains and backbone elements of the former are those that participate in the creation of pharmacophors and the present invention relates to the creation of such pharmacophors, or their mimics and their incorporation in the scaffolds of the invention for the purpose of creating a mimic of a region of the protein.

As mentioned above, the essential amino acids within a protein may be determined by the method of combinatorial alanine–scanning. Alanine scanning, a method of systematic and sequential alanine substitution, has been particularly useful for the identification of pharmacophors in a given peptide sequence. This method is based on the synthesis of a library in which each amino acid residue in a peptide chain is sequentially replaced by alanine, and biological screening of the library. Substitution of functional amino acid residues by the methyl group of alanine leads to the removal of all the side chain atoms past the β-carbon. Thus, the role of side-chain functional groups at specific position can be inferred. Alanine residue have the same backbone dihedral angles as other functional residues and thus the backbone conformation is not drastically perturbed by such substitution, as would be the case in glycine scan libraries. In this case, the side chain is nullified, which

leads to the introduction of flexibility into the peptide backbone.

An additional method for the elucidation of pharmacophors is the synthesis and biological screening of omission libraries. Omission libraries, based on a given peptide sequence is a library that contain all the possible peptides that compose the parent peptide. Omission library is divided into sequential and non sequential. In sequential omission library, amino acids are omitted from the carboxy– and amino- ends, whereas in non sequential library amino acids are omitted from the interior of the peptide sequence. Thus, sequential omission library based on a hexapeptide contains 2 pentapeptides, 3 tetrapeptides, 4 tripeptides and 5 dipeptides (total of 14 peptides). Non-sequential omission library based on a hexapeptide contains 4 pentapeptides, 18 tetrapeptides, 27 tripeptides and 9 dipeptides (total of 58 peptides). Beside information on essential pharmacophors, omission libraries can furnish shorter active peptides that will facilitate the design of libraries.

Once the pharmacophors are determined they are incorporated into the new compounds of the invention as one of the R substituents, to produce combinatorial libraries that will permit, after undergoing screening with suitable assays to screen for and to select candidates for a variety of uses in human medicine, veterinary medicine and in agriculture. Each compound may bind at least two pharmacophors, usually at least three pharmacophors and typically up to six, preferably up to five pharmacophors. Preferably the compound contain three to five such pharmacophors, attached to the scaffold either directly or through a linker. At times, when more then four pharmacophors have to be linked to a molecule, this can be achieved through binding the additional fifth, sixth etc. pharmacophors to the pharmacophors that are already connected to the scaffold.

In conclusion, the method of the invention utilizes spatial libraries that can generate novel leads for the disruption of protein:protein, protein:peptide, protein:cell membrane and protein:nucleic acid interactions, in animals and plants.

The pharmaceutical composition of the invention may be administered by any of the known administration routes, *inter alia*, oral, intravenous, intraperitoneal, intramuscular, subcutaneous, sublingual, intraocular, intranasal or topical administration routes. Appropriate unit dosage forms of administration include the forms for oral administration, such as tablets, capsules, powders, granulates and oral solutions or suspensions and the forms for sublingual and buccal administration, the forms for parenteral administration useful for a subcutaneous, intramuscular or intravenous injection, as well as the forms for rectal administration.

The carrier should be selected in accordance with the desired mode of administration and include any known components, e.g. solvents; emulgators, excipients, talc; flavors; colors, etc. The pharmaceutical composition may comprise, if desired, also other pharmaceutically-active compounds which are used to treat the disease, eliminate side effects or augment the activity of the active component.

In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are administered orally, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

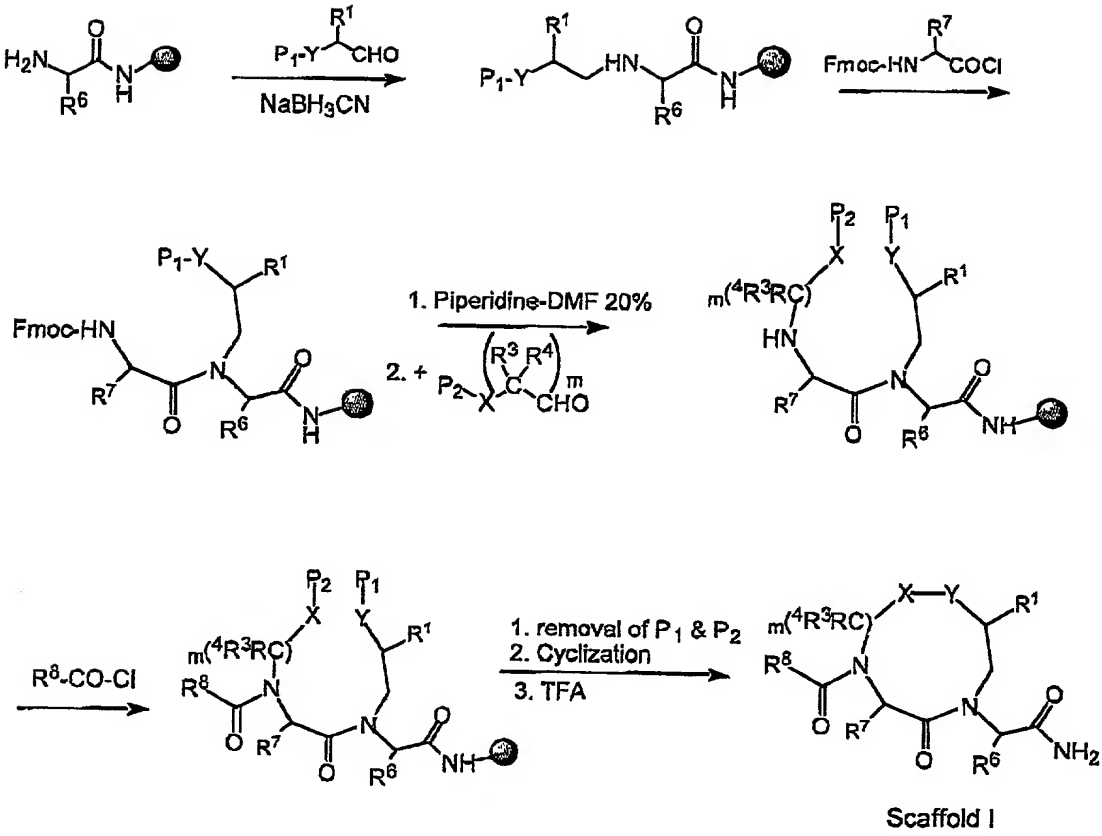
Typically, the pharmaceutical compositions of this invention will be administered from about 1 to about 5 times per day or alternatively, as a continuous infusion. A typical preparation will contain from about 5% to about 95% active compound (w1w). Preferably, such preparations contain from about 20% to about 80% active compound. As the skilled artisan will appreciate, lower or higher doses than those recited above may be required. Specific dosage and treatment regimens for any particular patient will depend upon a variety of factors, including the activity of the specific compound

employed, the age, body weight, general health status, sex, diet, time of administration, rate of excretion, drug combination, the patient's disposition to the disease state and the judgment of the treating physician. In general, the compound is most desirably administered at a concentration level that will generally afford effective results without causing any harmful or deleterious side effects.

The pharmaceutical composition may comprise, if desired, also other pharmaceutically-active compounds which are used to treat the disease, eliminate side effects or augment the activity of the active component.

Synthetic Approach

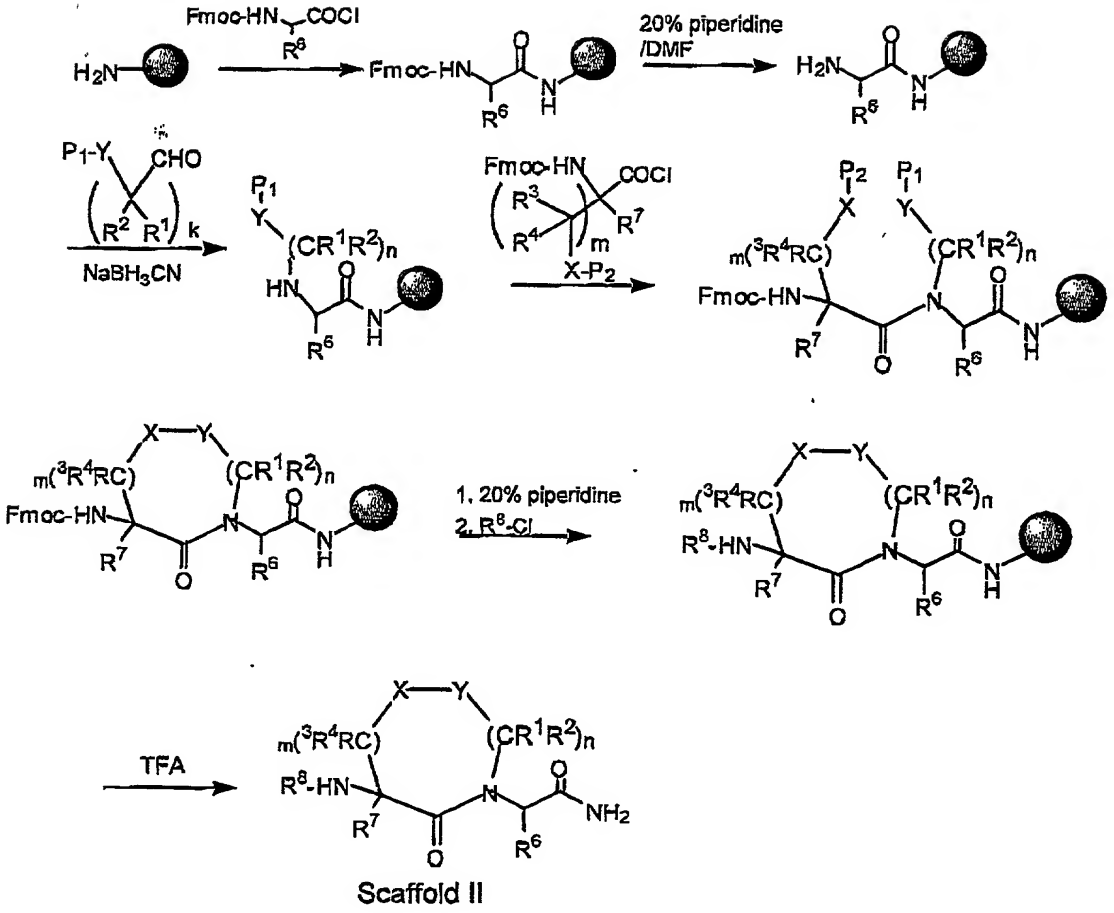
In general, the compounds of the invention are prepared according to the routes showed in Schemes 1-4 below. The solid phase synthesis of scaffolds I-IV comprise of a series of couplings of the appropriate protected acids and reductive alkylations with ω-functionalized protected aldehydes. The assembly of the appropriate linear scaffold on the solid support is followed by removal of the protecting groups P_1 and P_2 and cyclization. The appropriate scaffold is obtained after deprotection-removal from the solid support.



P_1 and P_2 are orthogonal protecting groups on Y and X respectivly

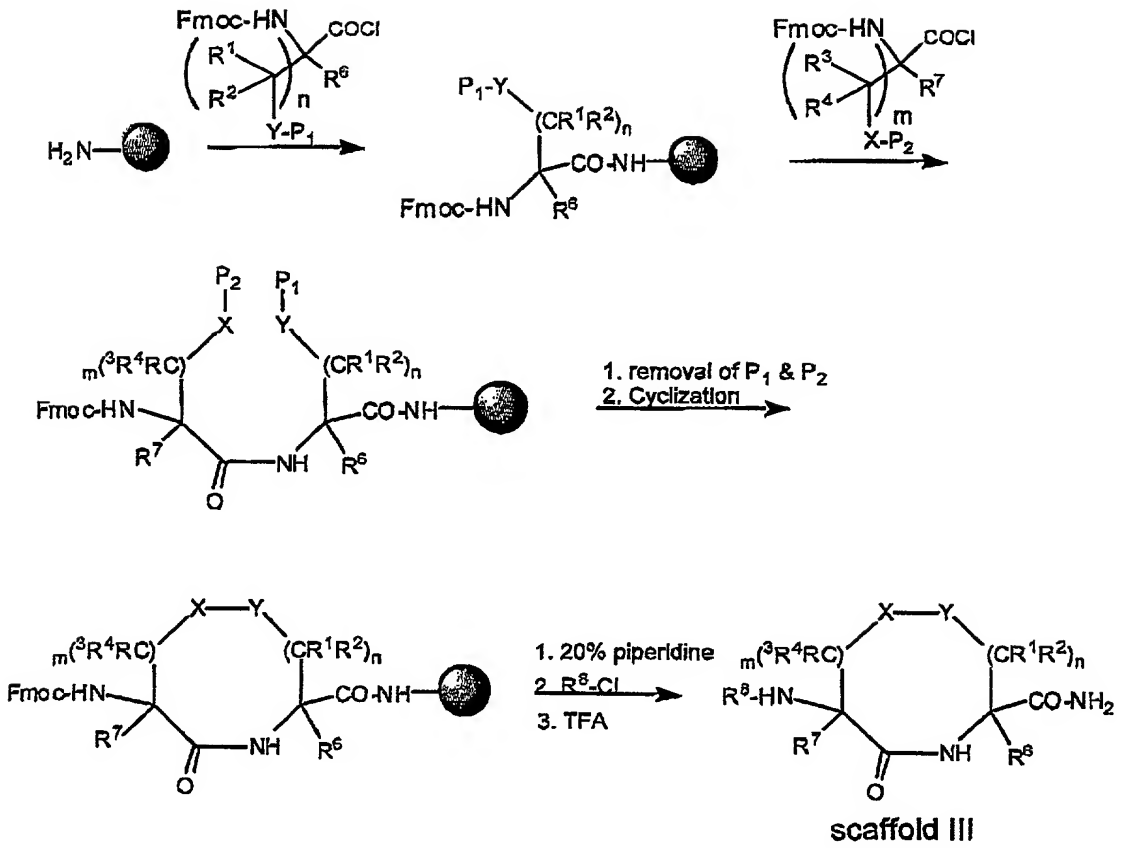
R^2 = H; L = NH_2; n = 2

Scheme 1: General solid phase synthesis of libraries of scaffold I



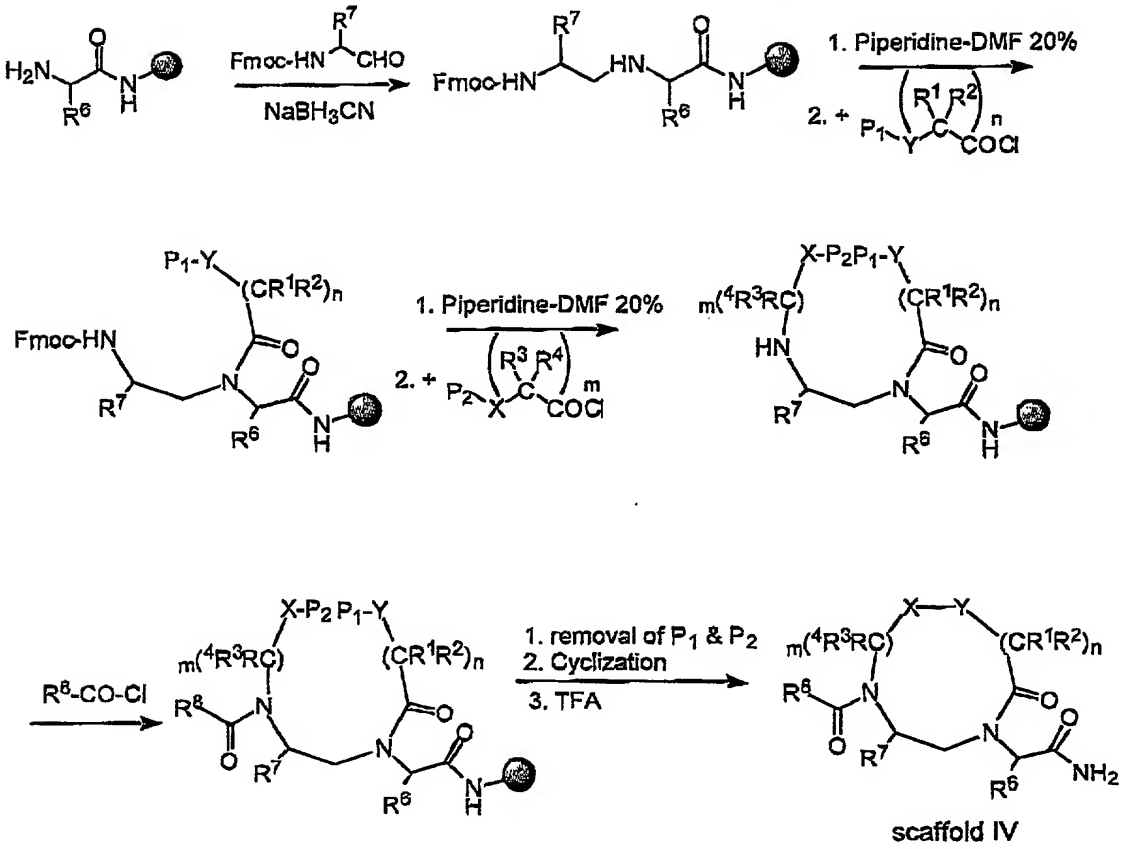
P_1 and P_2 are orthogonal protecting groups on Y and X respectivly

Scheme 2: General solid phase synthesis of libraries of scaffold II



P_1 and P_2 are orthogonal protecting groups on Y and X respectivly

Scheme 3: General solid phase synthesis of libraries of scaffold III



P_1 and P_2 are orthogonal protecting groups on Y and X respectivly

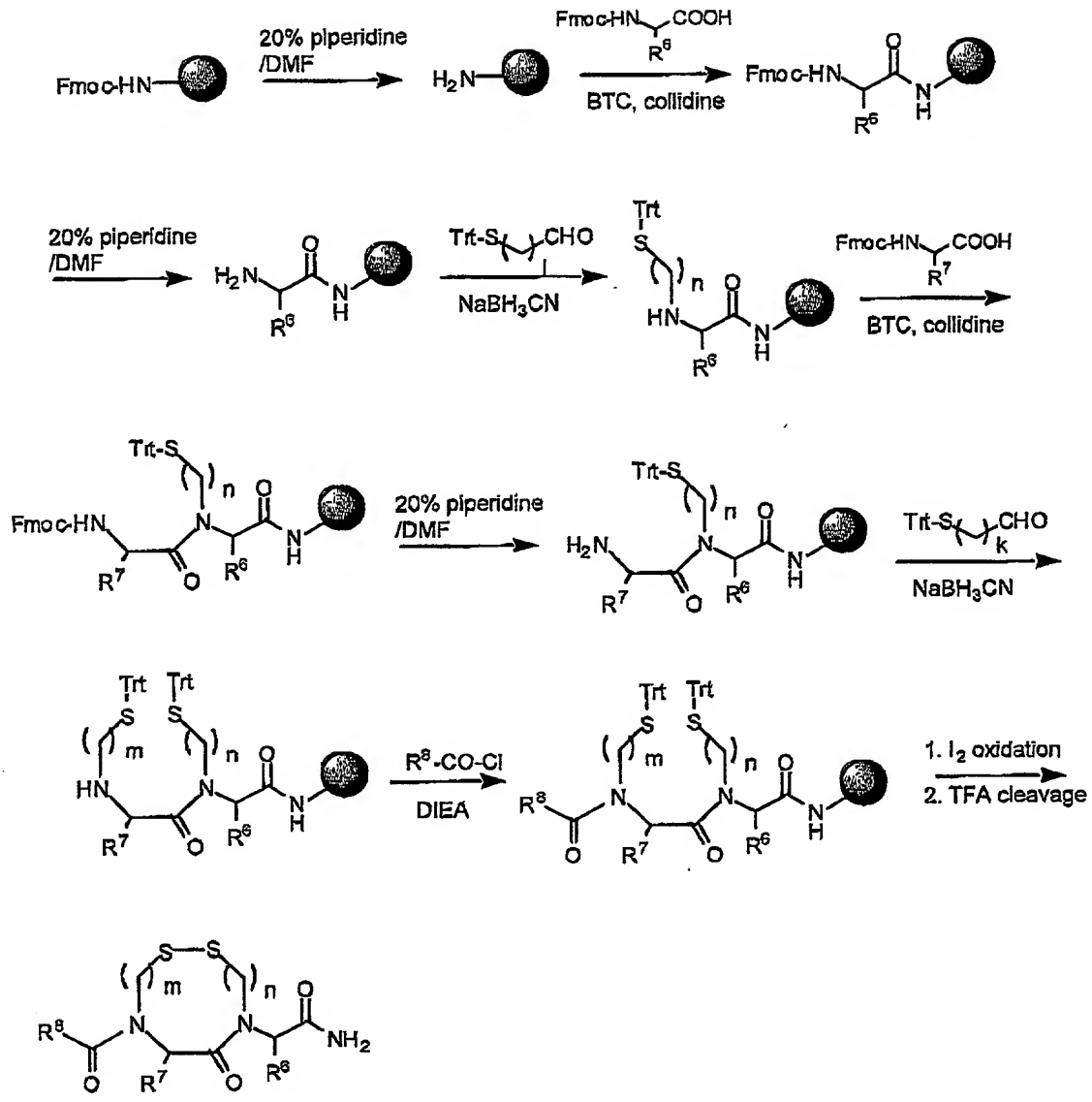
R^5 = H; L = NH_2

Scheme 4: General solid phase synthesis of libraries of scaffold IV

EXAMPLES

Synthesis

A library composed of 26 molecules that have the formula I and wherein R^1 and R^2 are either benzyl (side chain of phenylalanine) or hydroxybenzyl (side chain of tyrosine) and R^3 is benzyloxycarbonyl (which is a mimic of the side chain of phenylalanine) was synthesized and characterized. The library was synthesized by the Simultaneous Multiple Solid Phase methodology (Houghten (1985) *Proc. Natl. Acad. Sci. USA*, **82** 5131) as showed in Scheme 5 below. The molecules were characterized by HPLC, MS and MS-MS spectrometry.



Scheme 5: Solid phase synthesis of a library of compounds according to the invention (for structures of R^6, R^7, R^8, m and n, see Table 1)

Synthetic procedures according to Scheme 5 above:

Rink amide MBHA resin [Rink H. (1987)] (0.1g in each bag, 0.6 mmol/g) was preswollen for 2h in NMP while shaking in reaction vessel equipped with sintered glass bottom. The Fmoc protecting group was removed from the resin by reaction with 20% piperidine in NMP (2×30 min). Fmoc removal was monitored

by chloranil test. A coupling cycle was carried out with Fmoc-AA (AA is abbreviation of *amino acid*) (5eq), BTC (1.65eq), and 2,4,6 colidine (14eq) in DCM for 2h at room temperature. Reaction completion was monitored by qualitative chloranil test. Following coupling the peptidyl- resin was washed with DCM (×5) and for 2 min. Fmoc removal and washing steps were carried out as described above. Fmoc removal was monitored by the chloranil test.

The peptidyl-resin was then washed by a mixture of NMP: MeOH 1:1/ 1% and a solution of the aldehyde (1eq) in the mixture above was added (10 ml for 12 bags). Then additional 40 ml of this mixture was added and the mixture was shaken for 5 min. Then, 2eq of $NaBH_3CN$ were added and the reaction vessel was shaken for 2 h. The resin was washed as follows: DCM (2X2 min), EtOH (2X2 min), NMP (2X2 min), DCM (3X2 min). (Chloranil test gave blue color immediately). The following coupling was performed using Fmoc-AA (5eq), BTC (1.65 eq) and 2, 4, 6 colidine (14 eq) in dibromomethane at 50÷C for 2h and was repeated when necessary. Fmoc deprotection and washing steps were carried out as described above. Reductive alkylation and washing steps were carried out as described above. A solution of benzylchloroformate (6 eq), and DIEA (12 eq) in DMF was added to the resin and the mixture was shaken for 1h. The reaction was repeated and ten the resin was washed with NMP (5X2min) and DCM (2X2 min). Reaction completion was monitored by chloranil test.

Disulfide bridge formation:

The disulfide bridge was oxidized using iodine (10 eq) in DCM and shaking for 3h. The resin was washed as follows: DMF (2X2 min), 2% ascorbic acid in DMF (2X2 min), NMP (5X2 min), DCM (4X2 min).

Analytical procedures:

All the crude compounds were analyzed by MS and analytical reversed-phase HPLC (RP18 Vydak 4×250 mm; flow: 1 mL/min; T=30°C; detection UV 214 nm; gradient: A=0.1% TFA in TDW, B=0.1% TFA in CH_3CN,

0 min 95:5, 5 min 95:5, 33 min 5:95, 38 min 95:5, 42 min 95:5).

The molecules were purified by preparative reversed-phase HPLC (RP18 Vydak 2.5x250 mm; flow: 9 mL/min; T=30°C; detection UV 214 nm; gradient: A=0.1% TFA in TDW, B=0.1% TFA in CH_3CN, 0 min 95:5, 5 min 95:5, 33 min 5:95, 38 min 95:5, 42 min 95:5).

Fractions were collected, lyophilized and characterized by analytical HPLC and MS analysis. Results are shown in Table 1 below.

Synthesis of aldeydes

Trityl thiopropanal:

a) Trityl- thiopropanoic acid

Trityl mercaptan (36.13 g, 0.131 mol) was added stepwise to a suspension of NaH (11.5 g, 60% in mineral oil 0.288 mol) in 80 mL DMF under cooling and nitrogen atmosphere, the reaction mixture was stirred 30 minutes after the addition was completed. Then, a solution of bromopropionic acid (20 g, 0.131 mol) dissolved in 50 mL DMF was added stepwise. After the addition was completed the reaction mixture was stirred for 30 minutes and then cooling and nitrogen atmosphere were stopped and the reaction mixture was sealed and left overnight. Then, 500 mL chloroform were added and the mixture was washed with 4x200mL of saturated solution of $KHSO_4$ and 4x200 mL TDW (the solid that precipitate during the washings should also be collected with the organic layer). The organic layer was evaporated and the product (that contained DMF traces) was precipitated by adding 300 mL TDW and stirring for few minutes. The product was collected by filtration and dried by suction and then in *vaccuo*. The crude product was purified as follows: 150mL of $CHCl_3$ were added to the white solid and the mixture was stirred for few minutes. Then 200mL of PE 40-60 were added and the solid was collected by filtration yielding 37.61 g (82% yield) of white powder, mp 177-183°C, ^1H NMR ($CDCl_3$, 300MHz, 298K) δ 2.24 (t, 2H), 2.46 (t, 2H), 7.18-7.48 (m, 15H). MS (ES) m/z 347.

b) Trityl- thiopropanoic acid hydroxamate

A solution of N,O dimethylhydroxylamine hydrochloride (2.188 g, 0.0225 mol) in 40 mL DMF was added to a mixture of 6.96 g (0.02 mol) of Trityl-thiopropanoic acid and PyBoP (11.45 g, 0.022 mol). DIEA (10.4 mL, 0.06 mol) was added and the clear solution was stirred for 3 hours. EA (120 mL) was added to the stirred solution followed by 240 mL of saturated bicarbonate solution. The organic layer was collected and washed with additional two portions of 100 mL of saturated bicarbonate solution, 100 mL of TDW, 2×100 mL $KHSO_4$ 1M, and 100 mL TDW, dried over Na_2SO_4 and evaporated to dryness yielding 10.36 g (92% yield) of yellow oil. ^{1}H NMR ($CDCl_3$, 300MHz) δ 2.38 (t, 2H), 2.51 (t, 2H), 3.10 (s, 3H), 3.56 (s, 3H), 7.15-7.50 (m, 15H).

c) Trityl- thiopropanal

$LiAlH_4$ (2.014 g, 0.053 mol) was added in portions to a solution of 10.36 g (0.0265 mol) of Trityl- thiopropanoic acid hydroxamate in 260 mL dry diethyl ether under cooling in ice bath and argon atmosphere. The reaction mixture was stirred for 2 hours (monitored by TLC PE:EA=1:1). 560 mL of EA were added followed by addition of 560 mL of $KHSO_4$ 1M. The mixture was stirred for additional 30 minutes. The organic layer was collected and washed with 390 mL of $KHSO_4$ 1M and 390 mL of saturated NaCl, dried over Na_2SO_4 and evaporated yielding 7.68 g (87% yield) of white solid. ^{1}H NMR ($CDCl_3$, 300MHz, 298K) δ 2.36 (t, 2H), 2.46 (t, 2H), 7.15-7.50 (m, 15H), 9.55 (t, 1H).

Trityl thiobutyral

a) Trityl-thiobutyric acid:

Trityl mercaptan (36.13 g, 0.131 mol) was added stepwise to a suspension of NaH (11.5 g, 60% in mineral oil 0.288 mol) in 100 mL DMF under cooling and nitrogen atmosphere, the reaction mixture was stirred 30 minutes after the addition was completed. Then, a solution of bromobutyric acid (21.88 g, 0.131 mol)

dissolved in 150 mL DMF was added stepwise. After the addition was completed the reaction mixture was stirred for 30 minutes and then cooling and nitrogen atmosphere were stopped and the reaction mixture was sealed and left overnight. Then, 500 mL chloroform were added and the mixture was washed with 4x200mL of saturated solution of $KHSO_4$ and 4x300 mL of water (the solid that precipitate during the washings should also be collected with the organic layer). The organic layer was evaporated and the oily product (that contained DMF traces) was triturated by adding 300 mL TDW and stirring vigorously for few minutes. The product was collected by filtration, washed by TDW and dried by suction. The crude product was purified as follows: 200 mL of PE was added to the white solid and the mixture was stirred for 15 minutes. The solid was collected by filtration and dried in *vaccuo* yielding 36.82 g (77.6% yield) of white powder. ^{1}H NMR ($CDCl_3$, 300MHz, 298K) δ 1.67 (m, 2H), 2.22 (t, 2H), 2.30 (t, 2H), 7.10-7.50 (m, 15H).

b) Trityl- thiobutyric acid hydroxamate

A solution of N,O dimethylhydroxylamine hydrochloride (0.83 g, 0.0084 mol) in 20 mL DMF was added to a mixture of 2.77 g (0.0076 mol) of Trityl-thiobutyric acid and PyBoP (4.39 g, 0.0084 mol). DIEA (4 mL, 0.023 mol) was added and the clear solution was stirred for 3 hours (pH should be monitored and kept basic). EA (50 mL) was added to the stirred solution followed by 90 mL of saturated bicarbonate solution. The organic layer was collected and washed with additional two portions of 40 mL of saturated bicarbonate solution, 40 mL of water, 2x40 mL $KHSO_4$ 1M, and 40 mL water, dried over Na_2SO_4 and evaporated to dryness yielding 3.06g (quantitative yield) of yellow oil. ^{1}H NMR ($CDCl_3$, 300MHz) δ 1.74 (m, 2H), 2.23 (t, 2H), 2.38 (t, 2H), 3.13 (s, 3H), 3.63 (s, 3H), 7.10-7.50 (m, 15H).

c) Trityl- thiobutanal

$LiAlH_4$ (0.574 g, 0.0151 mol) was added in portions to a solution of 3.06 g (0.0075 mol) of the hydroxamate in 100 mL dry diethyl ether under cooling in ice bath and argon atmosphere. The reaction mixture was stirred for 1 hour (monitored by TLC PE:EA=1:1). 150 mL of EA were added followed by addition of 150 mL of $KHSO_4$ 1M. The mixture was stirred for additional 30 minutes. The organic layer was collected and washed with 100 mL of $KHSO_4$ 1M and 100 mL of saturated NaCl, dried over Na_2SO_4 and evaporated yielding 1.90 g (73% yield) of white solid. ^1H NMR ($CDCl_3$, 300MHz, 298K) δ 1.66 (m, 2H), 2.22 (t, 2H), 2.38 (t, 2H) 7.15-7.50 (m, 15H), 9.61 (t, 1H).

Trityl thiovaleric aldehyde

a) Trityl-thiovaleric acid:

Trityl mercaptan (36.13 g, 0.131 mol) was added stepwise to a suspension of NaH (11.5 g, 60% in mineral oil 0.288 mol) in 100 mL DMF under cooling and nitrogen atmosphere, the reaction mixture was stirred 30 minutes after the addition was completed. Then, a solution of bromovaleric acid (23.71 g, 0.131 mol) dissolved in 150 mL DMF was added stepwise. After the addition was completed the reaction mixture was stirred for 30 minutes and then cooling and nitrogen atmosphere were stopped and the reaction mixture was sealed and left overnight. Then, 500 mL chloroform were added and the mixture was washed with 4x200mL of saturated solution of $KHSO_4$ and 4x300 mL of water (the solid that precipitate during the washings should also be collected with the organic layer). The organic layer was evaporated resulting in a solid product (that contained DMF traces). 300 mL TDW were added and the mixture was stirred vigorously for few minutes. The product was collected by filtration and partially dried by suction. The crude product was purified as follows: 200 mL of PE was added to the white solid and the mixture was stirred for a few minutes. The solid was collected by filtration and dried in *vaccuo* yielding 45.06 g (91% yield) of white powder. ^1H NMR ($CDCl_3$,

300MHz, 298K) δ 1.41 (m, 2H), 1.58 (m, 2H), 2.18 (m, 4H), 7.15-7.45 (m, 15H). MS (ES) m/z 376.

b) Trityl- thiovaleric acid hydroxamate

A solution of N,O dimethylhydroxylamine hydrochloride (0.7 g, 0.0071 mol) in 16 mL DMF was added to a mixture of 2.45 g (0.0065 mol) of Trityl-thiovaleric acid and PyBoP (3.73 g, 0.0071 mol). DIEA (3.4 mL, 0.02 mol) was added and the clear solution was stirred for 3 hours (pH should be monitored and kept basic). EA (50 mL) was added to the stirred solution followed by 90 mL of saturated bicarbonate solution. The organic layer was collected and washed with additional two portions of 40 mL of saturated bicarbonate solution, 40 mL of water, 2×40 mL $KHSO_4$ 1M, and 40 mL water, dried over Na_2SO_4 and evaporated to dryness yielding 3.06g (quantitive yield) of yellow oil. ^1H NMR ($CDCl_3$, 300MHz) δ 1.74 (m, 2H), 2.23 (t, 2H), 2.38 (t, 2H), 3.13 (s, 3H), 3.63 (s, 3H), 7.10-7.50 (m, 15H).

c) Trityl thiovaleric aldehyde

$LiAlH_4$ (0.574 g, 0.0151 mol) was added in portions to a solution of 3.06 g (0.0075 mol) of the hydroxamate in 100 mL dry diethyl ether under cooling in ice bath and argon atmosphere. The reaction mixture was stirred for 1 hour (monitored by TLC PE:EA=1:1). 150 mL of EA were added followed by addition of 150 mL of $KHSO_4$ 1M. The mixture was stirred for additional 30 minutes. The organic layer was collected and washed with 100 mL of $KHSO_4$ 1M and 100 mL of saturated NaCl, dried over Na_2SO_4 and evaporated yielding 1.90 g (73% yield) of white solid. ^1H NMR ($CDCl_3$, 300MHz, 298K) δ 1.66 (m, 2H), 2.22 (t, 2H), 2.38 (t, 2H) 7.15-7.50 (m, 15H), 9.61 (t, 1H).

Trityl thiohexanal:

a) Trityl-thiohexanoic acid:

Trityl mercaptan (36.13 g, 0.131 mol) was added stepwise to a suspension of NaH (11.5 g, 60% in mineral oil 0.288 mol) in 100 mL DMF under cooling and nitrogen atmosphere, the reaction mixture was stirred 30 minutes after the addition was completed. Then, a solution of bromohexanoic acid (25 g, 0.128 mol) dissolved in 150 mL DMF was added stepwise. After the addition was completed the reaction mixture was stirred for 30 minutes and then cooling and nitrogen atmosphere were stopped and the reaction mixture was sealed and left overnight. Then, 500 mL chloroform were added and the mixture was washed with 4×200mL of saturated solution of $KHSO_4$ and 4×300 mL of water (the solid that precipitate during the washings should also be collected with the organic layer). The organic layer was evaporated and the product (that contained DMF traces) was triturated by adding 300 mL TDW and stirring vigursly for few minutes. The product was collected by filtration, washed by TDW and dried by suction. The crude product was purified as follows: The solid was dissolved in a mixture of 150 ml of $CHCl_3$ and 200 ml of PE, and the solution was evaporated. The oil obtained was triturated by addition of 100 ml PE and 50 ml of Et_2O then 50 ml Et_2O and 50 ml PE. The solid was collected by filtration and dried in *vaccuo* yielding 33.92 g (68% yield) of white powder. ^1H NMR ($CDCl_3$, 300MHz, 298K) δ 1.31 (m, 2H), 1.37 (m, 2H), 1.50 (m, 2H), 2.15 (t, 2H), 2.26 (t, 2H), 7.15-7.50 (m, 15H).

b) Trityl- thiohexanoic acid hydroxamate

A solution of N,O dimethylhydroxylamine hydrochloride (1.23g, 0.0126 mol) in 25 mL DMF was added to a mixture of 4.47g (0.0115 mol) of Trityl-thiohexanoic acid and PyBoP (6.56 g, 0.0126 mol). DIEA (6 mL, 0.0344 mol) was added and the clear solution was stirred for 3 hours (pH should be monitored and kept basic). EA (70 mL) was added to the stirred solution followed by 130 mL of saturated bicarbonate solution. The organic layer was collected and washed with additional two portions of 60 mL of saturated bicarbonate solution, 60 mL of water, 2×60 mL $KHSO_4$ 1M, and 60 mL water, dried over Na_2SO_4 and evaporated to dryness yielding 4.29 g (86% yield) of yellow oil. ^1H NMR ($CDCl_3$, 300MHz) δ 1.29 (m, 2H), 1.44 (m, 2H), 1.51 (m, 2H), 2.16 (t, 2H), 2.33 (t, 2H), 3.15 (s, 3H), 3.65 (s, 3H) 7.15-7.50 (m, 15H).

c) Trityl- thiohexanal

$LiAlH_4$ (0.75 g, 0.0198 mol) was added in portions to a solution of 4.29 g (0.0099 mol) of the hydroxamate in 130 mL dry diethyl ether under cooling in ice bath and argon atmosphere. The reaction mixture was stirred for 1 hour (monitored by TLC PE:EA=1:1). 200 mL of EA were added followed by addition of 200 mL of $KHSO_4$ 1M. The mixture was stirred for additional 30 minutes. The organic layer was collected and washed with 140 mL of $KHSO_4$ 1M and 140 mL of saturated NaCl, dried over Na_2SO_4 and evaporated yielding 3.45 g (93% yield) of white solid. ^1H NMR ($CDCl_3$, 300MHz, 298K) δ 1.38 (m, 2H), 1.49 (m, 2H), 1.60 (m, 2H), 2.15 (t, 2H), 2.34 (t, 2H), 7.10-7.55 (m, 15H), 9.71 (t, 1H).

Trityl thioacetaldehyde and trityl thiopentanal were prepared by procedures similar to those described above.

Table 1: structure and MS characterization of a library according to the present invention, the preparation of which is showed in Scheme 5 above.

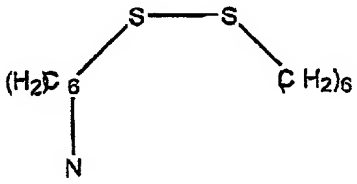
compound number	R^6	R^7	R^8	m	n	M.W calc.	M.W Obsvd.
1	L-hydroxybenzyl	L-Benzyl	Z#	4	5	649.87	653.3
2	L-hydroxybenzyl	L-Benzyl	Z	5	4	649.87	653.3
3	L-Benzyl	D-hydroxybenzyl	Z	4	5	649.87	653.2
4	L-Benzyl	D-hydroxybenzyl	Z	5	4	649.87	653.3
5	D-hydroxybenzyl	L-Benzyl	Z	4	5	649.87	653.2
6	D-hydroxybenzyl	L-Benzyl	Z	5	4	649.87	653.2
7	D-Benzyl	L-hydroxybenzyl	Z	4	5	649.87	653.3
8	D-Benzyl	L-hydroxybenzyl	Z	5	4	649.87	653.2
9	L-hydroxybenzyl	D-Benzyl	Z	4	5	649.87	653.2*
10	L-hydroxybenzyl	D-Benzyl	Z	5	4	649.87	653.3
11	L-hydroxybenzyl	L-hydroxybenzyl	Z	6	6	707.94	711.79
12	D-hydroxybenzyl	D-hydroxybenzyl	Z	6	6	707.94	711.85
13	L-hydroxybenzyl	D-hydroxybenzyl	Z	6	6	707.94	711.91
14	D-hydroxybenzyl	L-hydroxybenzyl	Z	6	6	707.94	711.43
15	L-hydroxybenzyl	L-Benzyl	Z	6	6	691.94	694.8
16	D-hydroxybenzyl	D-Benzyl	Z	6	6	691.94	693.41
17	L-hydroxybenzyl	D-Benzyl	Z	6	6	691.94	693.79
18	D-hydroxybenzyl	L-Benzyl	Z	6	6	691.94	693.91
19	L-Benzyl	L-hydroxybenzyl	Z	6	6	691.94	693.79
20	D-Benzyl	D-hydroxybenzyl	Z	6	6	691.94	693.6
21	L-Benzyl	D-hydroxybenzyl	Z	6	6	691.94	693.23**
22	D-Benzyl	L-hydroxybenzyl	Z	6	6	691.94	693.23**
23	L-Benzyl	L-Benzyl	Z	6	6	675.95	677.24**
24	D-Benzyl	D-Benzyl	Z	6	6	675.95	677.23**
25	L-Benzyl	D-Benzyl	Z	6	6	675.95	N.D
26	D-Benzyl	L-Benzyl	Z	6	6	675.95	677.30**

Z = benzyloxy carbonyl

* The peak was obtained relatively with low intensity.

** The peak was analyzed with HRMS.

Mass spectrometric anlysis: The discrepancy between the calculated and the observed mass as described in Table 1 ranges between 1.5 to 2.5 amu. These results may indicate the existence of reduced non cyclic molecule rather then the oxidized desired macrocycles. In order to negate this possibility the peaks were analyzed by splitting (marked with ** in Table 1). This analysis yielded the expected MW values with a discrepancy of only 0.3 amu. Furthermore, these molecules were also analyzed by MS-MS and a fragment indicating a disulfide bridge was found:



$C_{12}H_{24}NS2$
Exact Mass: 246.14
Mol. Wt.: 246.46